The Bio-Normalizer Clinical Efficacy Against Lead Intoxication

Final Report

Clinical Trial

"The Bio-Normalizer Clinical Efficacy Against Lead Intoxication"

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ABSTRACT

A Phase II double-blind case-controlled randomized clinical study of Japanese health food supplement "Bio-Normalizer" (BN) has been performed in manual workers with chronic lead intoxication due to their occupational exposure to lead-containing materials. 40 lead-bearing patients with clinical, hematological and biochemical symptoms of intoxication were randomized into 4 groups: the group IA patients were given 3g BN a day, group IB patients were given 6g BN a day, group IC patients were given 9g BN a day, and the group II patients (control) were given placebo daily for 3 weeks. We found that the blood lead content slightly increased and urine lead excretion decreased in the Group IA patients together with a decrease in the content of δ-aminolevulenic acid (ALA). Along with that, the clinical conditions of IA group patients improved significantly. The group IB patients exhibited the increased level of blood lead but lead excretion with urine did not show any changes. At the same time, the ALA and CP levels diminished. However, the Group B patients had complaints to painfulness in the extremities that was possibly explained by a fast evacuation of lead from the bone storage into the blood stream. A slight decrease in the blood lead level was found in the Group C patients; the levels of ALA and CP insignificantly decreased and increased, respectively. All the parameters of lead bearing and porphyrin metabolism were not significantly changed in the control group patients. On the basis of the results obtained, we concluded that BN may be regarded as a non-toxic natural chelator capable of mobilizing lead from the organism's storage, removing it from organism, and improving the impaired porphyrin metabolism. The optimal schemes and dosages of the BN application have to be evaluated individually.

INTRODUCTION

Lead is an environmental toxin causing hematological, gastrointestinal, and neurological dysfunctions. The sources of lead that are damaging for human health, arise from the industrial and other technological uses of lead such as lead mining, smelting, and refining, electric storage battery manufacturing, printing, glass making, and so on. The major dispersive non-recoverable use of lead is in the manufacture and application of alkyllead fuel additives. The highest exposure occurs in manual workers who come into contact with lead-containing materials during mining, smelting, or manufacturing processes. Other environmental sources of lead exposure are soil, dust, food, drinking water, paint, cosmetics, etc. Various miscellenous sources of lead, for example, lead-glazed ceramics used for beverage storage and illicitly-distilled whisky have been identified as highly hazardous.

The general features of lead absorbtion, distribution, metabolism, and excretion have been defined from both animal and human studies. The major pathway of exposure is inhalation, therefore more than 35% of lead being deposited in the lungs [1, 2]. A mechanism of lead absorption from pulmonary deposition is poorly understood. Meanwhile, there is some evidence that alveolar macrophages contribute to lead transportation into blood stream and tissues. About 10% of total lead content are derived from food and beverages and absorbed in gastrointestinal tract. It becomes evident that there are two general pools of lead within an organism: a comparatively large and slow-turnover pool and a smaller more rapidly-metabolizing pool. The first one is mainly located in bones and the second one consists of the soft tissues including the blood. Lead stored

principally in the bone tissue cell nuclei, microsomes, lysosomes, and mitochondria. It has been shown that calcium channels are responsible for the incorporation of lead in mitochondria [3]. As a consequence, lead in bone accumulates through most of the life span. The concentration of lead in the blood is of prime importance in the evaluation of lead exposure. This parameter is relied upon either to confirm the diagnosis of poisoning or to assess hazardous conditions both in occupationally-exposed people and in the general population. It has long been established that lead circulating in the blood is mainly bound to the erythrocytes, namely, to hemoglobin [4,5]. The nature of such an association remains obscure. Elimination of lead from the body is mainly by urine excretion (more than 75%). Alkyllead fuel additives (tetraethyllead and tetramethyllead) are dealkylated in liver and the lungs giving an inorganic lead.

Studies of the effects of lead on man have shown that it causes an increased mortality due to cerebrovascular diseases and chronic nephritis. Lead exposure results in the numerous disturbances in hematopoietic system. The effects of inorganic lead on the central nervous system have been under intensive investigation in recent years and clearly showed that classical lead encephalopathy occurs together with hyperactivity and other behavioural disturbances. On cellular and molecular levels, lead slows nerve condition, alters calcium homeostasis, inhibits enzymes, and stimulates synthesis of binding proteins. Lead is able to form complexes with thiols, phosphates, and organic acids. The most important mode of its toxic action, which takes place the lowest lead concentrations, is the suppression of heme synthesis by the inhibition of δ-aminolevulenic acid dehydrogenase. It in its turn leads to the elevation of erythrocyte protoporphyrin IX, increase in urinary δ-aminolevulenic acid and coproporphyrin excretion, the inhibition of erythrocyte Na+-K+-

ATPase, and decrease in hemoglobin level. This anaemic state is clearly an indication of lead adverse effects. Besides, the enhancement of the level of δ-aminolevulenic acid and the appearence of coproporphyrin in urine is one of the most important biochemical marker of lead intoxication. A decrease in heme synthesis results in the reduction of level of cytochrome P-450 [2]. In addition, lead affects significantly some liver functions for example enhancing the aminoacid transferase activities in serum. Thus, rats received lead acetate with drinking water exhibited a highly increased activities of alanin- and aspartate amine transferase in serum [6]. It is believed that an increase in the activities of cytosolic enzymes is typical for the action of heavy metals on the membrane integrity and permeability. At the same time, lead exposure suppressed aspartate amine transferase activity in rat kidneys [7]. Lead nitrate irreversibly inhibited types A and B monoamine oxygenase in bovine brain mitochondria [8].

The molecular and cellular mechanisms of lead toxicity remain unclear. It is thought that lead toxic effects mainly depend on the competition between calcium and lead for the binding sites. The replacement of calcium by lead in calmodulin may result in a variety of disturbances in the cellular functions[9]. However, at present, free radical-mediated mechanism of lead-induced damaging effects is one of the leading hypothesis. Indeed, Lawton and Donaldson [10] have shown that lead exposure led to enhancement of lipid peroxidation in the liver microsomes. Similar results were obtained by Ribarov et al. [11] in isolated erythrocytes. An increase in lipid peroxidation upon lead administration to rats has also been shown in brain [12,13]. Donaldson and Knowles [14] have reviewed the significance of free radical-mediated mechanisms in lead toxicity and concluded that these mechanisms may be involved in a majority of lead-induced adverse effects. Since early

thirties, researchers and physicians have been looking for reliable methods and medical preparations potent to increase the elimination of lead from the human body and to diminish its adverse effects on man's health. Unfortunately, most of known drugs capable of ameliorating the lead toxicity are highly toxic themselves, that does not allow to apply them at appropriate dosage for a long-term treatment. Therefore, it is important to discover new naturally occurring non-toxic compounds which can suppress pathological effects of lead poisoning.

A main goal of present study was to evaluate the clinical efficacy of natural non-toxic food supplement "Bio-Normalizer" in patients poisoning with lead due to their occupational duties.

Patients.

40 patients of both sexes aged from 30 to 55 years were admitted to Clinical Department of Ukranian Institute of Occupational Health. All of them had been in occupational contact with lead-containing materials not more than 10 years. They were manual workers in radio industry, printing, glass and electric charge battery manufacturing, and liquidators of the catastrophy on Chernobyl atomic power station. Patients eligible for this study were persons with clinically and laboratory proven Pb-bearing and/or symptomes of chronic lead intoxication. Among the clinical symptoms there were weakness, pale skin, paraesthesia and painfulness in the extremities, painfulness and the enlargement of liver, headache and stomachache; a very specific lead border of the gums was also observed. The diagnosis of occupational lead intoxication was confirmed by analytical, biochemical and hematological

methods. Patients with uremia, alcoholism, serious liver disorders, and a poor compliance were ineligble.

Study design.

Eligible patients were randomised to receive either conventional therapy plus Bio-Normalizer or conventional therapy plus placebo. The placebo resembled the BN sachet in size, colour, shape and taste. The study was double-blinded with both patients and treating physicians unaware of which study food additive was given. The method of randomization was based on tables of random numbers.

Treatment plan.

Patients of both arms received a concomitant therapy daily. In addition, patients received either BN or a placebo orally daily during 21 days. Experimental group was subdivided into 3 subgroups depending on scheme of BN administration: the group IA patients were given 3g of BN daily at bedtime, the group IB patients were given 6g of BN a day at bedtime, and the group IC patients were given 9g of BN a day (3g of BN in the morning before breakfast and 6g of BN at bedtime). The control group patients were given 3g of sugar powder at bedtime. The patients of both arms were frequently followed-up by a therapist and 3 times examined with conventional analytical, biochemical, and hematological analyses.

Ethical considerations.

The protocol complied with the European guidelines on human experimentation and the protocol was reviewed and approved by the Institute of Occupational Health's Ethics Committee. All patients were informed about the goals and methods of present clinical trial and gave written consent prior to randomization.

Study parameters.

Lead content in the patients' blood and urine was measured by standard flame atomic absorption method with slight modifications [14]. Biochemical effects of lead were determined by measurement of urinary δ-aminolevulenic acid and coproporphyrin levels. The classic method of Mauzerall and Granick [15] was used for urinary d-ALA determination. To measure coproporphyrins in urine, porphyrins were extracted by ethylacetate-acetic acid following by transferring the extract into hydrochloric acid. Then, absorbance at wave length 401 nm was determined [16]. Conventional biochemical assays were used for the protein, glucose, bilirubin, urea, creatinin, cholesterol, and β-lipoprotein determination as well as for measurement of ALT, AST, and GGT activities.

Statistical methods.

All results are presented as average±SD. Differences were analyzed using the Student's *t*-test, the level of significance being set at $P < 0.05$.

RESULTS AND DISCUSSION

At the beginning of the trial, an average lead content in the patients of control group (II) was equal to 0.607 mg/L, and it practically did not change to the trial end (0.612 mg/L). Daily urine excretion of lead was about 0.1 mg. ALA content in urine was equal to 3.75 to 17.8 μM/g creatinine (average of 6.2 μM/g) and also did not change during the trial. Coproporphyrin and creatinine contents were stable. Thus, the 21 days administration of placebo did not affect the lead blood content and lead excretion. Patients' conditions were practically without any change.

Another situation was observed when BN was administrated to patients of Group I. Lead blood content in the patients of Groups IA and IC changed insignificantly, being equal to about 0.5 mg/L, while there was a significant increase in this parameter during the trial for the Group IB patients (from 0.424 to 0.740 mg/L). Urine excretion of lead slightly decreased in the patients of Group IA and remained without any change in Groups IB and IC. ALA and coproporphyrin contents were stable. Patients' conditions in Group I were improving (a better sleep, good spirits, etc.). There were no essential changes in morphological and biochemical blood composition.

Patients of Group 1B with chronic lead intoxication suffered from polyneuropathy, chronic colitis, chronic hepatitis, and normochromic anemia. As it was already mentioned out, in this group lead blood content increased from 0.424 to 0.740 mg/L without significant change in lead excretion with urine. Simultaneously, there was a decrease in the contents of ALA and coproporphyrin. Clinical conditions of patients remained satisfactory: 4 patients showed significant improvement. Some variations in hemoglobin and

erythrocytes observed in 6 patients. Similar to the Group IA patients, certain variations in lipoprotein contents were observed for the patients of this group. However, the Group B patients had complaints to painfulness in the extremities that was possibly explained by a fast evacuation of lead from the bone storage into the blood stream.

The patients of IC Group were characterized by a slight decrease in lead blood level. Lead excretion with urine did not change. Other parameters were stable. Clinical conditions of patients were significantly improved. An increase in hemoglobin level was observed for some patients, which characterized by its diminished initial content.

CONCLUSIONS

1. The consumption of BN was not associated with any adverse effects as it is evident from vital signs and a variety of clinico-chemical parameters for liver, renal, and cardio-vascular functions and the blood cell count (See Tables 1-20).

2. Administration of 3g BN a day to the patients of Group IA enhanced both the lead blood level and urine lead excretion. Simultaneously, there was a decrease in the level of δ-aminolevulenic acid. Thus, the administration of even the smallest BN doses improved the parameters characterized lead intoxication. This conclusion corresponds to the improvement of patients' clinical conditions.

3. Administration of 6g BN a day to the patients of Group IB significantly increased the lead blood level and diminished ALA and coproporphyrin contents, parameters determined the lead-induced damage. Thus, this dosage seems to be the most efficient one in combating the consequences of lead intoxication. However, the Group IB patients had

complaints to painfulness in the extremities probably due to the fast lead evaculation from the bone storage to the blood stream.

4. Administration of 9g BN a day to the patients of Group IC led to a slight decrease in lead blood level without any change in the other parameters. Clinical conditions of patients were significantly improved.

5. In accord with the above findings, we concluded that BN may be considered as a non-toxic natural chelator capable of mobilizing lead from the organism's storage, removing it from an organism, and improving the impaired porphyrin metabolism. The optimal schemes and dosages of BN application have to be evaluated individually.

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Table 1 Lead and iron content in the blood of BN-treated patients (Group IA)

N (patient)	Pb (μmol/L) 1st visit	Pb (μmol/L) 3d visit	Fe (μmol/L) 1st visit	Fe (μmol/L) 3d visit
1 (001)	1.36	2.12		
2 (003)	2.61	2.12		
3 (005)	3.67	3.91		
4 (009)	0.48	4.68		
5 (011)	3.86	2.90		
6 (013)	2.06	2.65		
7 (014)	2.99	2.46		
8 (015)	2.99	2.50		
9 (020)	1.01	1.89	14.5	14.35
10 (021)	3.04	2.94		
Average	2.41±0.94	2.82±0.63		

Table 2. Lead and iron content in the blood of BN-treated patients (Group IB)

N (patient)	Pb (μmol/L) 1st visit	Pb (μmol/L) 3d visit	Fe (μmol/L) 1st visit	Fe (μmol/L) 3d visit
1 (002)	2.80	2.27	25.07	37.24
2 (006)	1.40	4.73		
3 (007)	1.25	4.78		
4 (008)	0.82	4.73		
5 (010)	3.71	4.44		
6 (012)	3.67	2.99	28.47	43.5
7 (018)	0.92	2.03	17.55	
8 (019)	1.25	3.86		
9 (022)	2.70	3.04	12.9	14.3
10 (016)	1.83	2.75	15.76	
Average	2.04±0.95	3.56±0.95	20.0±5.5	31.7±12

Table 3. Lead and iron content in the blood of BN-treated patients (Group IC)

N (patient)	Pb (μmol/L) 1st visit	Pb (μmol/L) 3d visit	Fe (μmol/L) 1st visit	Fe (μmol/L) 3d visit
1 (004)	4.30	1.64		
2 (017)	4.10	2.94	32.4	
3 (023)	2.99	2.90	15.2	
4 (024)	2.90	3.60	49.4	26.7
5 (025)	2.32	4.15	13.1	
6 (026)	4.49	5.07	16.3	
7 (27)	3.61	3.04		16.5
8 (28)	3.62	2.7	21.1	19.2
9 (29)	3.72	3.37	25.8	
10 (30)	3.86	2.90		23.3
Average	3.59±0.51	3.23±0.65	24.8±9.5	21.4±3.6

Table 4 Lead and iron content in the blood of control patients (Group II)

N (patient)	Pb (μmol/L) 1st visit	Pb (μmol/L) 3d visit	Fe (μmol/L) 1st visit	Fe (μmol/L) 3d visit
1 (001)	2.22	2.90	34.4	19.0
2 (002)	1.59	2.36	27.6	53.5
3 (003)	2.03	2.22	10.0	34.4
4 (004)	2.36	3.43	53.5	
5 (005)	4.30	2.50	25.4	
6 (006)	3.43	3.52	37.8	20.4
7 (007)	3.09	3.10	22.7	22.7
8 (008)	3.80	2.81	24.2	24.2
9 (009)	3.30	3.52	30.4	17.2
10 (010)	3.38	2.81	6.7	17.4
Average	2.95+0.72	2.92+0.38	27.3+9.5	26.1+8.9

Table 5 Lead and creatinin content in urine of BN-treated patients (Group IA)

N (patient)	Pb μmol/L (1st visit)	Pb μmol/L (3d visit)	Creatinin mmol/L (1st visit)	Creatinin mmol/L (3d visit)	Pb mg/day (1st visit)	Pb mg/day (3d visit)
1 (001)	0.35	0.43	28.5	24.0	0.088	0.249
2 (003)	0.89	0. 29	9.5	11.0	0.208	0.180
3 (005)	0.22	0.14	19.4	20.0	0.073	0.170
4 (009)	0.43	0.43	17.8	15.8	0.143	0.122
5 (011)	0.50	0.25	19.9	10.3	0.147	0.058
6 (013)	0.41	0.43	22.5	11.4	0.085	0.170
7 (014)	0.35	0.47	8.62	8.44	0.146	0.203
8 (015)	0.54	0.35	19.6	12.0	0.165	0.131
9 (020)	0.33	0.27	14.6	24.8	0.101	0.082
10 (021)	0.54	0.29	32.2	26.8	0.124	0.150
Average	046±0.13	0.34±0.09	19.3±5.3	16.4±5.9	0.128± 0.034	0.152± 0.042

Table 6 Lead and creatinin content in urine of BN-treated patients (Group IB)

N (patient)	Pb μmol/L (1st visit)	Pb μmol/L (3d visit)	Creatinin mmol/L (1st visit)	Creatinin mmol/L (3d visit)	Pb mg/day (1st visit)	Pb mg/day (3d visit)
1 (002)	0.41	0.47	24.5	24.8	0.178	0.176
2 (006)	0.39	0.31	40.0	17.2	0.121	0.130
3 (007)	0.43	0.27	16.0	12.1	0.178	0.079
4 (008)	0.33	0.41	43.5	28.5	0.102	0.085
5 (010)	0.41	0.24	24.6	22.5	0.102	0.053
6 (012)	0.25	0.47	17.9	18.8	0.106	0.174
7 (016)	0.35	0.33	26.8	18.8	0.080	0.086
8 (018)	0.39	0.35	24.2	33.3	0.119	0.211
9 (019)	0.50	0.33	13.3	16.9	0.131	0.110
10 (022)	0.39	0.43	18.8	19.2	0.089	0.106
Average	0.39±0.05	0.36±0.07	25.0±7.1	21.2±4.9	0.121± 0.025	0.121± 0.41

Table 7 Lead and creatinin content in urine of BN-treated patients (Group IC)

N (patient)	Pb μmol/L (1st visit)	Pb μmol/L (3d visit)	Creatinin mmol/L (1st visit)	Creatinin mmol/L (3d visit)	Pb mg/day (1st visit)	Pb mg/day (3visit)
1 (004)	0.43	0.39	17.3	19.9	0.085	0.097
2 (017)	0.39	0.35	10.2	21.6	0.186	0.091
3 (023)	0.47	0.47	23.8	22.5	0.174	0.155
4 (024)	0.47	0.37	19.0	27.5	0.137	0.161
5 (025)	0.39	0.33	20.0	19.2	0.153	0.131
6 (026)	0.35	0.35	22.5	24.2	0.077	0.069
7 (027)	0.37	0.41	28.2	26.4	0.053	0.112
8 (028)	0.35	0.37	28.5	23.1	0.081	0.100
9 (029)	0.33	0.39	27.8	21.6	0.129	0.060
10 (030)	0.39	0.47	24.8	26.8	0.141	0.194
Average	0.39± 0.04	0.39± 0.04	22.2±4.5	23.3±2.4	0.122± 0.038	0.117± 0.034

Table 8 Lead and creatinin content in urine of control patients (Group II)

N (patient)	Pb μmol/L (1st visit)	Pb μmol/L (3d visit)	Creatinin mmol/L (1st visit)	Creatinn mmol/L (3d visit)	Pb mg/day (1st visit)	Pb mg/day (3d visit)
1 (001)	0.47	0.47	19.0	22.5	0.137	0.155
2 (002)	0.33	0.32	14.6	14.0	0.086	0.080
3 (003)	0.24	0.31	10.7	13.4	0.053	0.091
4 (004)	0.22	0.27	22.5	18.3	0.082	0.057
5 (005)	0.37	0.39	23.2	22.5	0.053	0.113
6 (006)	0.42	0.47	19.7	22.5	0.132	0.105
7 (007)	0.37	0.38	18.9	24.2	0.154	0.090
8 (008)	0.39	0.50	24.6	24.5	0.131	0.151
9 (009)	0.37	0.33	38.9	22	0.084	0.057
10 (010)	0.39	0.39	15.0	19.5	0.067	0.081
Average	0.36±0.06	0.38±0,06	20.7±5.3	20.3±3.2	0.098± 0.032	0.098± 0.026

Table 9 δ-aminolevulenic acid (ALA) and coproporphyrin contents in urine of BN-treated patients (IA)

N (patient)	ALA μmol/g creatinin (1st visit)	ALA μmol/g creatinin (3d visit)	Coproporphyrin mmol/g creatinin (1st visit)	Coproporphyrin mmol/g creatinin (3d visit)
1 (001)	2.72	2.12	12.4	64
2 (003)	3.94	5.94	38.6	125.8
3 (005)	4.57	8.73	91.2	91.3
4 (009)	4.03	3.12	135.1	105.0
5 (011)	20.5	4.3	138.3	196.6
6 (013)	18.5	4.2	22.2	177.5
7 (014)	1.28	3.21	88.7	160.3
8 (015)	20.0	5.68	95.6	115.8
9 (020)	5.39	4.69	49.2	54.3
10 (021)	6.54	4.85	77.7	77.0
Average	8.8±6.6	4.7±1.3	74.9±35.4	116±38.6

Table 10 δ-aminolevulenic acid (ALA) and coproporphyrin contents in urine of BN-treated patients (Group IB)

N (patient)	ALA μmol/g creatinin (1st visit)	ALA μmol/g creatinin (3d visit)	Coproporphyrin mmol/g creatinin (1st visit)	Coproporphyrin mmol/g creatinin (3d visit)
1 (002)	25.2	25.9	148.9	151.7
2 (006)	20.7	10.0	118.5	93.0
3 (007)	0.90	4.39	55.1	69.4
4 (008)	1.40	11.4	73.7	80.3
5 (010)	17.8	16.8	105.5	90.2
6 (012)	3.21	3.12	105.2	24.0
7 (016)	3.75	4.12	57.2	46.9
8 (018)	16.1	3.12	139.7	46.0
9 (019)	19.0	12.1	137.6	69.7
10 (022)	13.3	6.06	132.3	127.8
Average	12.1±7.8	9.7±5.5	107±28	80±29

Table 11 δ-aminolevulenic acid (ALA) and coproporphyrin contents in urine of BN-treated patients (Group IC)

N (patient)	ALA μmol/g creatinin (1st visit)	ALA μmol/g creatinin (3d visit)	Coproporphyrin mmol/g creatinin (1st visit)	Coproporphyrin mmol/g creatinin (3d visit)
1 (004)	3.56	4.03	54.6	130.9
2 (017)	18.0	16.3	97.6	104.7
3 (023)	21.8	14.1	155.8	151.6
4 (024)	17.8	11.8	109.1	105.3
5 (025)	3.79	6.44	47.4	82.9
6.(026)	10.2	8.71	130.9	90.4
7 (027)	5.68	14.1	72.7	133.7
8 (028)	8.71	8.71	87.8	100.7
9 (029)	10.15	10.0	91.7	112.9
10 (030)	10.23	16.3	156.4	94.2
Average	11.0±4.9	11.0±3.4	100±30	111±17

Table 12 δ-aminolevulenic acid (ALA) and coproporphyrin contents in urine of control patients (Group II)

N (patient)	ALA μmol/g creatinin (1st visit)	ALA μmol/g creatinin (3d visit)	Coproporphyrin mmol/g creatinin (1st visit)	Coproporphyrin mmol/g creatinin (3d visit)
1 (001)	17.8	14.1	109.1	151.6
2 (002)	4.03	4.0	86.7	86.6
3 (003)	4.48	5.12	93.1	96.2
4 (004)	4.76	6.44	151.6	69.9
5 (005)	16.1	17.9	139.1	132.3
6 (006)	9.30	14.09	109	151.6
7 (007)	5.57	6.84	76.4	72.1
8 (008)	7.80	8.39	88.5	144.4
9 (009)	3.75	4.92	90.2	82.7
10 (010)	7.73	3.12	99.2	79.9
Average	7.1±3.7	7.9±4.1	104±18	102±31

Table 13 Blood biochemistry in BN-treated patients (Group IA)

N	Sugar mmol/L 1st visit	Sugar mmol/L 3d visit	β-proteins (%)[a] 1st visit	βproteins (%)[a] 3d visit	ALT 1st visit	ALT 3d visit	AST 1st visit	AST 3d visit
1 (001)	4.6	5.2	42.0	80.8	0.61	0.66	0.35	0.40
2 (003)	6.6	5.3	44	43	0.37	0.41	0.16	0.36
3 (005)	4.6	5.6	65	58	0.33	0.37	0.24	0.33
4 (009)	4.2	5.0	68	35	0.29	0.37	0.16	0.20
5 (011)	5.6	4.7	58	4.9	0.34	0.33	0.24	0.24
6 (013)	4.7	3.3	44	52	0.29	0.26	0.20	0.16
7 (014)	4.8	5.2	80	60	0.29	0.29	0.24	0.16
8 (015)	5.0	3.8	65	90	0.22	0.41	0.16	0.24
9(020)	3.3	4.3	46	80	0.45	0.37	0.16	0.24
10(021)	7.2	12.5	50	78	0.29	0.41	0.28	0.24
m ± SD	5.1±0.8	5.5±1.4	56±11	58±20	0.349± 0.077	0.388± 0.068	0.219± 0.051	0.257± 0.064

a) Per cent of phospholipids

Table 14 Blood biochemistry in BN-treated patients (Group IB)

N	Sugar mmol/L 1st visit	Sugar mmol/L 3d visit	βproteins (%)[a] 1st visit	βproteins (%)[a] 3d visit	ALT 1st visit	ALT 3d visit	AST 1st visit	AST 3d visit
1 (002)	4.7	4.2	34	49	0.22	0.42	0.32	0.28
2 (006)	4.8	5.1	70	49	0.45	0.41	0.20	0.24
3 (007)	4.4	5.0	95	107	0.26	0.26	0.20	0.16
4 (008)	3.7	4.4	56	42	0.29	0.45	0.32	0.24
5 (010)	6.5	4.9	62	39	0.33	0.39	0.16	0.24
6 (012)			54	40	0.29	0.33	0.16	0.24
7 (016)	5.7	4.9	42	58	0.29	0.29	0.24	0.20
8(018)	5.4	4.9	64	52	0.45	0.49	0.16	0.20
9 (019)			44	60	0.29	0.41	0.28	0.24
10 (022)	3.4	3.7	34	33	0.33	0.45	0.20	0.28
Average	4.8±0.8	4.6±0.4	56±14	53±13	0.32 ±0.06	0.39± 0.06	0.22± 0.05	0.23± 0.03

Table 15 Blood biochemistry in BN-treated patients (Group IC)

N	Sugar mmol/L 1st visit	Sugar mmol/L 3d visit	β-proteins (%)[a] 1st visit	β-proteins (%)[a] 3d visit	ALT 1st visit	ALT 3d visit	AST 1st visit	AST 3d visit
1 (004)	3.7	5.5	70	54	0.26	0.45	0.45	0.40
2 (017)	3.6	4.6	38	34	0.54	0.33	0.66	0.24
3 (023)	3.8	4.1	46	40	0.33	0.29	0.20	0.20
4 (024)	4.8	4.4	80	78	0.41	0.42	0.24	0.27
5 (025)	3.7	4.2	72	39	0.37	0.29	0.20	0.20
6 (026)	3.4	5.0	35	40	0.41	0.33	0.32	0.32
7 (027)	4.7	3.3	36	36	0.45	0.32	0.24	0.20
8 (028)	3.7	4.0	78	80	0.26	0.45	0.24	0.24
9 (029)	3.6	3.5	37	44	0.26	0.29	0.12	0.32
10 (030)	5.3	4.8	70	78	0.26	0.30	0.32	0.42
Average	4.0±0.5	4.3±0.5	56±18	52±16	0.36± 0.08	0.35± 0.06	0.30± 0.11	0.28± 0.07

Table 16 Blood biochemistry in control patients (Group II)

N	Sugar mmol/L 1st visit	Sugar mmol/L 3d visit	β-proteins (%)[a] 1st visit	β-proteins (%)[a] 3d visit	ALT 1st visit	ALT 3d visit	AST 1st visit	AST 3d visit
1 (001)	4,1	4.4	54	58	0.33	0.40	0.32	0.30
2 (002)	5.6	4.8	60	47	0.46	0.49	0.28	0.28
3 (003)	4.5	4.2	55	49	0.45	0.49	0.16	0.37
4 (004)	4.8	4.6	52	44	0.53	0.41	0.40	0.20
5 (005)	4.0	4.3	42	34	0.41	0.41	0.16	0.36
6 (006)	4.2	4.1	58	60	0.40	0.42	0.24	0.28
7 (007)	4.2	3.7	64	56	0.41	0.33	0.28	0.28
8 (008)	4.0	3.6	44	33	0.49	0.26	0.28	0.40
9 (009)	3.4	4.2	37	38	0.37	0.34	0.20	0.30
10 (010)	4.8	4.1	48	50	0.32	0.45	0.20	0.36
Average	4.36	4.2	51.4	46.9	0.417	0.40	0.252	0.313
SD (±)	0.452	0.26	6.9	7.7	0.052	0.054	0.060	0.048

a) Per cent of phospholipids

Table 17 Anemia symptoms of BN-treated patients (Group IA)

N (patient)	Hb (g/L) 1st visit	Hb (g/L) 3d visit	RBC (10^{12}/L) 1st visit	RBC (10^{12}/L) 3d visit
1 (001)	138	134	4.0	4.20
2 (003)	149	140	4.72	4.50
3 (005)	63.5	70.3	2.12	1.82
4 (009)	113	113	3.5	3.2
5 (011)	113	114	3.46	3.50
6 (013)	113	122	3.68	3.76
7 (014)	120	120	3.5	3.5
8 (015)	139	130	4.5	4.6
9 (020)	116	120	3.7	3.7
10 (021)	126	126	3.84	4.0
Average	119±15	119±12	3.7±0.5	3.7±0.5

Table 18 Anemia symptoms of BN-treated patients (Group IB)

N (patient)	Hb (g/L) 1st visit	Hb (g/L) 3d visit	RBC (10^{12}/L) 1st visit	RBC (10^{12}/L) 3d visit
1 (002)	113	113	3.52	3.68
2 (006)	113	120	3.52	3.84
3 (007)	116	120	3.2	3.5
4 (008)	129	138	3.9	4.2
5 (010)	129	125	3.7	3.9
6 (012)	142	142	4.5	4.36
7 (016)	129	129	4.0	4.2
8 (018)	120	120	3.6	3.5
9 (019)	142	130	4.36	4.0
10 (022)	126	113	3.8	3.3
Average	126±8	125±8	3.8±0.3	3.8±0.3

Table 19 Anemia symptoms of BN-treated patients (Group IC)

N (patient)	Hb (g/L) 1st visit	Hb (g/L) 3d visit	RBC (10^{12}/L) 1st visit	RBC (10^{12}/L) 3d visit
1 (004)	106	115	3.2	3.5
2 (017)	96.7	115	2.9	3.46
3 (023)	109	113	3.4	3.2
4 (024)	116	118	3.4	3.4
5 (025)	150	142	4.72	4.70
6 (026)	109	112	3.0	3.1
7 (027)	116	118	3.3	3.5
8 (028)	129	128	3.9	3.8
9 (029)	139	142	4.0	4.0
10 (030)	130	129	3.9	3.9
Average	120±14	123±10	3.6±0.4	3.7±0.4

Table 20 Anemia symptoms of control patients (Group II)

N (patient)	Hb (g/L.) 1st visit	Hb (g/L.) 3d visit	RBC (10^{12}/L.) 1st visit	RBC (10^{12}/L.) 3d visit
1 (001)	137	129	4.2	4.7
2 (002)	133	136	4.0	4.1
3 (003)	129	128	4.0	3.9
4 (004)	149	149	4.5	4.0
5 (005)	133	134	4.1	4.1
6 (006)	139	132	4.8	4.1
7 (007)	136	140	4.1	4.4
8 (008)	116	118	3.5	3.5
9 (009)	126	124	3.8	3.7
10 (010)	136	140	4.0	4.0
Average	133.4±6.0	133±6.8	4.1±0.24	4.05±0.23